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⑧ Novel insulin derivatives.

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Description

The present invention relates to novel insulin derivatives having improved properties, to methods for their preparation and to preparations containing such novel insulin derivatives.

In the treatment of diabetes mellitus, many varieties of insulin preparations have been suggested and used. Even though improved insulin preparations have steadily been invented during the insulin era, there is still a need for insulin preparations with improved properties.

Acidic solutions of insulin have been used earlier, both as short-acting preparations and together with protamine and/or zinc as long-acting preparations. However, under ordinary circumstances the chemical stability of insulin at pH values below 4.5 is low, as formation of desamidoinsulins (Sundby, F., J Biol Chem 237 (1962), 3406 - 3411) and covalent dimers (Steiner et al., Diabetes 17 (1968), 725 - 736) takes place. In the pH range 4.5 - 6.5, insulin precipitates. Hence, in order to obtain soluble short-acting insulin preparations (by the addition of blood-flow enhancing agents) and long-acting insulin preparations (by the addition of protamine and/or zinc) an insulin stable at a low pH would be desirable.

One object of this invention is to provide insulin derivatives with improved properties.

A second object of this invention is to provide solutions of insulin derivatives having an improved stability.

A third object of this invention is to provide preparations of insulin derivatives with low or with no immunogenic activity.

A fourth object of this invention is to provide insulin preparations which are soluble at pH values from 2.0 to 8.0, preferably from 2.0 to 4.5 and from 6.5 to 8.0.

A fifth object of this invention is to provide solutions of insulin derivatives having an improved stability at pH values of 3-4.

A sixth object of this invention is to provide long-acting solutions of insulin derivatives.

The present invention relates to human insulin, porcine insulin, rabbit insulin and des(B30) human insulin wherein the A21 amino acid has been substituted by Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val or hSer.

Such compounds can be designated by the general formula I

INSUL-A21

|

B30

(I)

wherein INSUL represents des(A21).des(B30) human insulin and A21 represents one of the amino acids Ala, Arg, Asn, Glu, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val or hSer connected to Cys²⁶ in INSUL, and B30 represents hydrogen or one of the amino acids Ser, Ala or Thr connected to Lys^{B29} in INSUL.

It is known that during the acidic ethanol extraction of mammalian insulins many dimers are formed (Steiner) and, furthermore, monodesamidoinsulins are formed under acid conditions (Sundby).

It has now, surprisingly, been found that the formation of such undesired dimers is substantially reduced or almost eliminated when the insulin compound used is one of the above insulin derivatives wherein Asn^{A21} has been exchanged with one of the above-mentioned amino acids. This substitution also eliminates the formation of monodesamidoinsulins.

The novel insulin derivatives have the following advantages:

1) The formation of the immunogenic dimers, i.e. covalently linked insulin molecules linked either through the two A-chains, (AA) dimer, or through one A-chain and one B-chain, (AB) dimer, (Helbig, H.J., Deutsche Welfforschungsinstitut, dissertation, 1976) is substantially eliminated (a chromatographic fraction of crude porcine insulin, the b-component, containing the dimers was shown to be immunogenic in rabbits (Schlichtkrull et al., Horm Metab Res. Suppl. 5 (1974), 134 - 143)).

2) The stability of the novel insulin derivatives is so high that it will probably be possible to store preparations containing these novel insulin derivatives at room temperature for a long period of time.

5) In the pH range of 3-4, which is inappropriate for mammalian insulin because of chemical instability, useful solutions of insulin derivatives can be made in the presence of magnesium ions in concentrations of 0.005 M to 0.5 M, preferably with insulin derivatives of formula I wherein A21 is different from Gln.

6) It will be possible to prepare soluble, rapidly acting preparations containing the novel insulin derivatives by the addition of compounds which enhance the absorption.

7) It will be possible to prepare soluble, retarded preparations containing the novel insulin derivatives by the addition of zinc and/or protamine to acid solutions, i.e. solutions having a pH value in the range from 2.5 to 4.

8) It will be possible to prepare preparations containing the novel insulin derivatives having different profiles.

Compounds of formula I may be prepared by a transpeptidation reaction in which a biosynthetic precursor compound having the general formula II

INSUL-A21

I
X

(II)

wherein A21 is as defined above, and X is a bond, an amino acid residue or a peptide residue bridging the carboxyl group of Lys^{B29} to the amino group of Gly^{A1}, is reacted with an amino compound of the general formula III

Z-OR (III)

wherein Z is Thr, Ala or Ser wherein any hydroxy group may be protected, and R is a carboxy protecting group (e.g. methyl or tert-butyl), using trypsin or a trypsin-like enzyme as a catalyst in a mixture of water and organic solvents analogously as described in US patent specification No. 4,343,898, whereafter the carboxy protecting group and any hydroxy protecting group is removed. X may for example be a moiety of the formula IV

(Q)_q-K (IV)

wherein Q is a peptide chain and q amino acids, q is an integer from 0 to 33, K is Lys or Arg, and r is zero or one.

Compounds of the formula II may be prepared by a method similar to the method described in European patent application Nos. 163,529 and 214,826. By this method a DNA-sequence encoding a compound with the formula II is inserted into a suitable expression vector which, when transferred to a suitable yeast strain, is capable of expressing the desired compound with correctly positioning disulphide bridges. The product expressed is then isolated from the cells or the culture broth depending on whether it is secreted from the cells or not.

At neutral pH, compounds of formula I have the same charge as human insulin. In solution, compounds of formula I may be present as hexamers.

Examples of specific preferred compounds according to the invention are the following: Gly^{A1} human insulin, Ala^{A1} human insulin, Ser^{A1} human insulin, Thr^{A1} human insulin, hSer^{A21} human insulin, Gly^{A21} porcine insulin, Ala^{A1} porcine insulin, Ser^{A1} porcine insulin and Thr^{A1} porcine insulin.

Insulin preparations of this invention can be prepared by dissolving a compound of formula I in an aqueous medium at slightly acidic conditions, for example in a concentration of from 240 to 600 nmole/ml.

The aqueous medium can be made isotonic by the addition of sodium chloride, sodium acetate or glycerol.

If a protracted preparation is required the above mentioned isotonic agents can in part or completely be replaced by a zinc salt or a mixture of zinc salts at a concentration of up to 5 µg Zn²⁺ per mmole of insulin.

to 0.5 M, preferably above 0.05 M. The upper limit is somewhat arbitrary being chosen from the assumption that in some cases (e.g. for intraperitoneal infusion) some overstepping of isotonicity may be acceptable. According to a preferred embodiment of this invention the preparations contain magnesium ions in a concentration of from 0.08 M to 0.3 M.

It has furthermore been found that protracted - or further protracted - preparations of the insulin derivatives of this invention are obtained when protamine is added to the above mentioned preparations, i.e. the preparations containing no zinc ions and no magnesium ions, the preparations containing zinc ions and the preparations containing magnesium ions. The amount of protamine to be used is from 5% to 50%, preferably from 8% to 40%, more preferred from 10% to 30% on the basis of insulin (weight weight).

Insulin preparations with enhanced absorption properties can also be obtained by the addition of arginine or lysine to an aqueous solution of the insulin. The preferred concentration of these amino acids is from 0.01 M to 0.2 M.

The insulin preparations may further contain buffers such as acetate and citrate and preservatives such as phenol, m-cresol and methyl paraben. The pH of the solution is adjusted to the desired value and the insulin preparation is made sterile by sterile filtration.

Insulin solutions of this invention having a pH value in the range 3 - 6.2 may also be particularly useful for the purpose of infusion by means of pumps, because of a lack of insulin precipitation caused by carbon dioxide diffusion through catheters. Such precipitation has been observed occasionally with neutral infusion solutions, and is believed to be attributable to the lowering of the pH value caused by carbon dioxide.

The abbreviations used herein for the amino acid residues are those state in J.Biol.Chem. 243 (1968), 3558. The amino acids stated herein are in L configuration. Within the context of this invention the term insulin when used in a plural or generic sense is intended to encompass both naturally occurring insulins and insulin derivatives. Gly^{A21} human insulin is human insulin wherein Asn^{A21} has been exchanged by Gly and similarly for similar names.

The insulin preparations of this invention can be used in the treatment of diabetes. It is recommended that the dosage of the insulin preparations of this invention be selected by a physician similarly to the selection of the dosage of known insulin preparations for injection.

Any novel feature or combination of features described herein is considered essential to this invention.

Example 1

Preparation of Gly^{A21} Human Insulin

Gly^{A21} human insulin was prepared by transpeptidation of a compound which according to formula II can be formulated as



wherein the terminal Ala of the budging peptide is linked to the carboxyl group of Lys^{B30} and Lys is linked to the amino group of Gly^{A1}, with Thr-OMe (L-threonine methylester) followed by hydrolysis of the ester group with aqueous sodium hydroxide. Thus 100 mg of the compound of formula V was dissolved in 0.5 ml of 10 M acetic acid and 1 ml of 2 M Thr-OMe in N,N-dimethylacetamide was added. The mixture was cooled to 12°C. 10 mg of trypsin dissolved in 0.2 ml of 0.05 M calcium acetate was added. After 48 hours at 12°C the proteins were precipitated by addition of 20 ml of acetone. The conversion of the starting material into Gly^{A21}-(Thr-OMe)^{B30} human insulin was 88% by HPLC.

250 mg of Gly^{A21}-(Thr-OMe)^{B30} human insulin was suspended in 25 ml of water and dissolved by the addition of 1 N sodium hydroxide solution to a pH value of 10.0. The pH value is kept constant at 10.0 for 24 hours.

The compound of formula V was prepared by a method analogous to example 2 of European patent application No. 214.826.

Example 2

Preparation of Injectable Solution of Compounds of Formula I

15 μ mol of Gly^{A21} human insulin containing 0.5% of zinc are dissolved in water (5 ml) containing hydrochloric acid (80 μ l of 1 N) followed by the addition of an aqueous solution (10 ml) containing phenol (65 mg) and glycerol (400 mg). The pH value of the solution is adjusted to 3.0 by means of a sodium hydroxide solution and the total volume is adjusted to 25 ml with water. The resulting solution is sterilized by filtration and subsequently transferred aseptically to vials (5 ml).

Example 3

Soluble Preparation of Gly^{A21} Human Insulin with Protracted Action

15 μ mol of Gly^{A21} human insulin (zinc free) are dissolved in water (5 ml). To this solution is added hydrochloric acid (80 μ l of 1 N) and zinc chloride (100 μ l of 0.6 M) followed by the addition of an aqueous solution (15 ml) containing protamine sulphate (37 mg), m-cresol (50 mg) and sodium chloride (200 mg). The pH is adjusted to 3.5 with sodium hydroxide solution and the total volume is adjusted to 25 ml with water. Finally, the solution is sterilized by filtration and transferred aseptically to sterile vials.

The absorption profile after subcutaneous injection in pigs was found comparable to that of the well known insulin suspension Protaphane® HM 100 IU/ml.

Example 4

Soluble Preparation of Gly^{A21} Human Insulin with Fast Action

15 μ mol of Gly^{A21} human insulin (zinc free) are dissolved in water (10 ml). To this solution is added hydrochloric acid (40 μ l of 1 N) and magnesium chloride (2.6 ml of 1 M) followed by the addition of an aqueous solution of benzyl alcohol (8 ml of 0.3 M). The pH is adjusted to 5.7 with sodium hydroxide solution and the total volume is adjusted to 25 ml with water. Finally the solution is sterilized by filtration and transferred aseptically to sterile vials.

Example 5

Chemical Stability of Gly^{A21} Human Insulin in Preparations

Three preparations containing 0.24 mM of Gly^{A21} human insulin (zinc free), 0.26% (w/v) of phenol and 1.6% (w/v) of glycerol were prepared and their pH value adjusted to 3.0, 4.0, and 5.0, respectively.

Samples were analyzed after storage at 45°C for two weeks using human insulin preparations of the same composition as reference.

Table 1 shows the content of insulin dimerization and polymerization products as determined by HPSEC (High Performance Size Exclusion Chromatography).

Table 2 shows the content of insulin deamidation products determined by DISC PAGE (Poly Acrylamide Gel Electrophoresis).

Table 1

pH of Preparation	Human Insulin	Gly ^{A21} Human Insulin
3.0	4.9%	0.31%
4.0	41.6%	1.0%
5.0	16.1%	2.8%
Dry Insulin	0.29%	0.05%

Table 2

pH of Preparation	Human Insulin	Gly ^{A21} Human Insulin
3.0	90%	2%
4.0	40%	3%
5.0	3%	4%
Dry Insulin	0.5%	0.5%

Example 6

Biological Potency of Gly^{A21} Human Insulin

Investigation according to the British Pharmacopoeia, 1980 edition, of the potency of Gly^{A21} human insulin showed that this was approximately 85% of that of human insulin. Within the dose range relevant for therapeutic purposes no toxic manifestations were observed.

solution (15 ml) containing protamine sulphate (37 mg), m-cresol (50 mg) and magnesium chloride (200 mg). The pH is adjusted to 3.5 and the total volume is adjusted to 25 ml with water. Finally, the solution is sterilized by filtration and transferred aseptically to sterile vials.

The absorption of this preparation after subcutaneous injection in pigs was found to be substantially slower than that of the well known insulin suspension Protaphane® HM 100 IU/ml.

The features disclosed in the foregoing description and in the following claims may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Insulin derivatives of the general formula I

INSUL-A21

|

(I)

B30

wherein INSUL represents des(A21), des(B30) human insulin, characterized in that A21 represents one of the amino acids Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val or hSer connected to Cys^{A20} in INSUL, and B30 represents hydrogen or one of the amino acids Ser, Ala or Thr connected to Lys^{B29} in INSUL, and preferably A21 is different from Phe.

2. Insulin derivatives according to Claim 1, wherein A21 represents Gly, Ala, Ser, Thr, or hSer, and B30 represents Ala or Thr.
3. Preparation characterized in that it contains a compound of formula I stated in Claim 1 or 2 above with the definitions stated therein.
4. Preparation according to Claim 3, characterized in that it is soluble.
5. Preparation according to Claim 4, characterized in that it contains a compound which enhances the absorption.
6. Preparation according to claim 5, characterized in that said compound is a magnesium salt.
7. Preparation according to claim 6, characterized in that it is a solution with a pH value in the range of 3-4 and that it contains magnesium ions in a concentration of 0.005 M to 0.5 M which preparation preferably contains a compound of formula I wherein A21 is different from Gln.
8. Preparation according to claim 5, characterized in that said compound is arginine or lysine.
9. Preparation according to Claim 3, 4, 6, 7 or 8 characterized in that it contains zinc ions and/or potassium.
10. Preparation according to any one of Claims 3 to 9, characterized in that it has a pH value in the range of from 2.0 to 8, preferably from 2.5 to 8.
11. Preparation according to any one of Claims 3 to 9, characterized in that it has a pH value in the range of from 2.5 to 4.5 or from 6.5 to 8.0.

INSUL-A21

|

X

(II)

wherein A21 is as defined in claim 1, and X is a bond, an amino acid residue or a peptide residue bridging the carboxyl group of Lys^{B29} to the amino group of Gly^{A1}, is reacted with an amino compound of the general formula III

Z-OR (III)

wherein Z is Thr, Ala or Ser wherein any hydroxy group may be protected, and R is a carboxy protecting group, using trypsin or a trypsin-like enzyme as a catalyst in a mixture of water and organic solvents whereafter the carboxy protecting group and any hydroxy protecting group is removed

13. Method according to claim 12, wherein X is a moiety of the formula IV

-(Q_r-K)_r (IV)

wherein Q is a peptide chain with q amino acids, q is an integer from 0 to 33, K is Lys or Arg, and r is zero or one

Claims for the following Contracting States : ES, GR

1. A method for the preparation of insulin derivatives of the general formula I

INSUL-A21

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B30

(I)

wherein INSUL represents des(A21), des(B30) human insulin, and A21 represents one of the amino acids Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val or hSer connected to Cys^{A17} in INSUL, and B30 represents hydrogen or one of the amino acids Ser, Ala or Thr connected to Lys^{B29} in INSUL, and preferably A21 is different from Phe, wherein a biosynthetic precursor compound having the general formula II

INSUL-A21

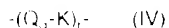
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X

(II)

wherein A21 is as defined above, and X is a bond, an amino acid residue or a peptide residue bridging the carboxyl group of Lys^{B29} to the amino group of Gly^{A1}, is reacted with an amino compound of the general formula III

2. Method according to Claim 1, wherein X is a moiety of the formula IV



wherein Q is a peptide chain with q amino acids, q is an integer from 0 to 33, K is Lys or Arg, and r is zero or one.

3. A method according to Claim 1 or 2, wherein A21 represents Gly, Ala, Ser, Thr or hSer, and B30 represents Ala or Thr.

4. Use of an insulin derivative of the general formula I



wherein INSUL represents des(A21), des(B30) human insulin, and A21 represents one of the amino acids Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val or hSer connected to Cys^{A20} in INSUL, and B30 represents hydrogen or one of the amino acids Ser, Ala or Thr connected to Lys^{B34} in INSUL, and preferably A21 is different from Phe in a method of manufacturing a preparation for the treatment of diabetes mellitus

5. Use according to Claim 4, wherein the preparation to be manufactured is soluble.
6. Use according to Claim 5, wherein the preparation to be manufactured contains a compound which enhances the absorption.
7. Use according to Claim 6, wherein said compound is a magnesium salt.
8. Use according to Claim 7, wherein the preparation to be manufactured is a solution with a pH value in the range of 3-4 and contains magnesium ions in a concentration of 0.005 M to 0.5 M, which preparation preferably contains a compound of formula I wherein A21 is different from Gln.
9. Use according to Claim 6, wherein said compound is arginine or lysine.
10. Use according to Claim 4, 5, 7, 8 or 9, wherein the preparation to be manufactured contains zinc ions and/or protamine.
11. Use according to any one of Claims 4 to 10, wherein the preparation to be manufactured has a pH value in the range of from 2.0 to 8, preferably from 2.5 to 8.
12. Use according to any one of Claims 4 to 10, wherein the preparation to be manufactured has a pH value in the range of from 2.5 to 4.5 or from 6.5 to 8.0.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Insulinderivate der allgemeinen Formel I



wobei INSUL des(A21) des(B30)-Humaninsulin darstellt, dadurch gekennzeichnet, daß A21 eine der Aminosäuren Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val oder hSer darstellt, die an das Cys^{A20} in INSUL gebunden sind, und B30 Wasserstoff oder eine der Aminosäuren Ser, Ala oder Thr darstellt, die an das Lys^{B29} in INSUL gebunden sind, und bevorzugt ist A21 von Phe verschieden.

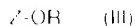
2. Insulinderivat nach Anspruch 1, wobei A21 Gly, Ala, Ser, Thr oder hSer darstellt und B30 Ala oder Thr darstellt.
3. Präparat, dadurch gekennzeichnet, daß es eine Verbindung der Formel I, die oben in den Ansprüchen 1 oder 2 mit den darin angegebenen Definitionen angegeben ist, enthält.
4. Präparat nach Anspruch 3, dadurch gekennzeichnet, daß es löslich ist.
5. Präparat nach Anspruch 4, dadurch gekennzeichnet, daß es eine Verbindung enthält, die die Absorption verbessert.
6. Präparat nach Anspruch 5, dadurch gekennzeichnet, daß die Verbindung ein Magnesiumsalz ist.
7. Präparat nach Anspruch 6, dadurch gekennzeichnet, daß es eine Lösung mit einem pH-Wert im Bereich von 3 bis 4 ist und daß es Magnesiumionen in einer Konzentration von 0,005 M bis 0,5 M enthält, wobei das Präparat bevorzugt eine Verbindung der Formel I enthält, wobei A21 von Gln verschieden ist.
8. Präparat nach Anspruch 5, dadurch gekennzeichnet, daß die Verbindung Arginin oder Lysin ist.
9. Präparat nach Anspruch 3, 4, 6, 7 oder 8, dadurch gekennzeichnet, daß es Zinkionen und oder Protamin enthält.
10. Präparat nach einem der Ansprüche 3 bis 9, dadurch gekennzeichnet, daß es einen pH-Wert in dem Bereich von 2,0 bis 8, bevorzugt von 2,5 bis 8, hat.
11. Präparat nach einem der Ansprüche 3 bis 9, dadurch gekennzeichnet, daß es einen pH-Wert in dem Bereich von 2,5 bis 4,5 oder von 6,5 bis 8,0 hat.
12. Verfahren zum Herstellen von Insulinderivaten nach Anspruch 1, bei dem eine biosynthetische Präkursor-Verbindung mit der allgemeinen Formel II

INSUL-A21



(II),

wobei A21 wie in Anspruch 1 definiert ist und X eine Bindung, ein Aminosäurerest oder ein Peptidrest ist, die von der Carboxylgruppe von Lys^{B29} zu der Amino-Gruppe von Gly^{A21} überbrückt, mit einer Amino-Verbindung der allgemeinen Formel III



reagiert wird, wobei Z Thr, Ala oder Ser ist, wobei jegliche Hydroxy-Gruppe geschützt werden kann, und R eine Carboxylschützende Gruppe ist, welche Trypsin oder ein Trypsin-Gleiches Enzym auf

ist, wobei Q eine Peptidkette mit q Aminosäuren ist, q eine ganze Zahl von 0 bis 33 ist, K Lys oder Arg ist und r Null oder Eins ist.

4. Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zum Herstellen von Insulinderivaten der allgemeinen Formel I

INSUL-A21

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B30

(I),

wobei INSUL des(A21), des(B30)-Humaninsulin darstellt und A21 eine der Aminosäuren Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val oder hSer darstellt, die an Cys^{A20} in INSUL gebunden sind, und B30 Wasserstoff oder eine der Aminosäuren Ser, Ala oder Thr darstellt, die an Lys^{B29} in INSUL gebunden sind, und bevorzugt ist A21 von Phe verschieden, bei dem eine biosynthetische Präkursor-Verbindung mit der allgemeinen Formel II

INSUL-A21

|

X

(II),

wobei A21 wie oben definiert ist und X eine Bindung, ein Aminosäurerest oder ein Peptidrest ist, der die Carboxylgruppe von Lys^{B29} zu der Aminogruppe von Gly^{A1} überbrückt, mit einer Aminoverbindung der allgemeinen Formel III

Z-OR (III)

reagiert wird, wobei Z Thr, Ala oder Ser ist, wobei jegliche Hydroxygruppe geschützt werden kann, und R eine Carboxy-schützende Gruppe ist, wobei Trypsin oder ein Trypsin-ähnliches Enzym als Katalysator in einer Mischung aus Wasser und organischen Lösemitteln verwendet wird, wonach die Carboxy-schützende Gruppe und jegliche Hydroxy-schützende Gruppe entfernt wird.

2. Verfahren nach Anspruch 1, bei dem X ein Anteil der Formel IV

-(Q₁-K)_r- (IV)

ist, wobei Q eine Peptidkette mit q Aminosäuren ist, wobei q eine ganze Zahl von 0 bis 33 ist, K Lys oder Arg ist und r Null oder Eins ist.

3. Verfahren nach Anspruch 1 oder 2, bei dem A21 Gly, Ala, Ser, Thr oder hSer darstellt und B30 Ala oder Thr darstellt.

4. Verwendung eines Insulinderivates der allgemeinen Formel I

wobei INSUL des(A21), des(B30)-Humaninsulin darstellt und A21 eine der Aminosäuren Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val oder hSer darstellt, die an Cys^{A20} in INSUL gebunden sind, und B30 Wasserstoff oder eine der Aminosäuren Ser, Ala oder Thr darstellt, die an Lys^{B29} in INSUL gebunden sind, und bevorzugt ist A21 von Phe verschieden, in einem Verfahren zum Herstellen eines Präparates für die Behandlung von Diabetes mellitus

5. Verwendung nach Anspruch 4, wobei das herzustellende Präparat löslich ist.
6. Verwendung nach Anspruch 5, wobei das herzustellende Präparat eine Verbindung enthält, die die Absorption verbessert.
7. Verwendung nach Anspruch 6, wobei die Verbindung ein Magnesiumsalz ist.
8. Verwendung nach Anspruch 7, wobei das herzustellende Präparat eine Lösung mit einem pH-Wert in dem Bereich von 3 bis 4 ist und Magnesiumionen in einer Konzentration von 0,005 M bis 0,5 M enthält, wobei das Präparat bevorzugt eine Verbindung der Formel I enthält, wobei A21 von Gln verschieden ist.
9. Verwendung nach Anspruch 6, wobei die Verbindung Arginin oder Lysin ist.
10. Verwendung nach Anspruch 4, 5, 7, 8 oder 9, wobei das herzustellende Präparat Zinkionen und oder Protamin enthält.
11. Verwendung nach einem der Ansprüche 4 bis 10, wobei das herzustellende Präparat einen pH-Wert in dem Bereich von 2,0 bis 8, bevorzugt von 2,5 bis 8 hat.
12. Verwendung nach einem der Ansprüche 4 bis 10, wobei das herzustellende Präparat einen pH-Wert im Bereich von 2,5 bis 4,5 oder von 6,5 bis 8,0 hat.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Dérivés de l'insuline de la formule générale I

INSUL-A21

|

(I)

B30

dans laquelle INSUL représente l'insuline humaine des(A21) des(B30), caractérisés en ce qu'A21 représente l'un des acides aminés Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val ou hSer relié à Cys^{A20} dans INSUL et B30 représente l'hydrogène ou l'un des acides aminés Ser, Ala ou Thr relié à Lys^{B29} dans INSUL, et de préférence A21 est différent de Phe.

2. Dérivés de l'insuline de la revendication 1, dans lesquels A21 représente Gly, Ala, Ser, Thr ou hSer et B30 représente Ala ou Thr.
3. Préparation caractérisée en ce qu'elle contient un composé de formule I désigné dans la revendication 1 ou 2 ci-dessus avec les définitions énoncées dans celle-ci.
4. Préparation selon la revendication 3, caractérisée en ce qu'elle est soluble.

9. Utilisation selon la revendication 6, dans laquelle le composé est l'arginine ou la lysine
10. Utilisation selon les revendications 4, 5, 7, 8 ou 9 dans laquelle la préparation à fabriquer contient des ions de zinc et/ou la pretamine
11. Utilisation selon l'une quelconque des revendications 4 à 10, dans laquelle la préparation à fabriquer possède une valeur de pH dans la plage allant de 2.0 jusqu'à 8, de préférence de 2.5 jusqu'à 8
12. Utilisation selon l'une quelconque des revendications 4 à 10, dans laquelle la préparation à fabriquer possède une valeur de pH dans la plage allant de 2.5 jusqu'à 4.5 ou de 6.5 jusqu'à 8.0